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UTILITY PATENT APPLICATION TRANSMITTAL (Only for new nonprovisional applications under 37 CFR 1.53(b))		
	Attorney Docket No.	CWP-012CN3
	First Named Inventor	Charlton
	Title	TEST DEVICE AND METHOD FOR COLORED PARTICLE IMMUNOASSAY

APPLICATION ELEMENTS	ADDRESS TO: Box Patent Application Assistant Commissioner for Patents Washington, D.C. 20231
1. ☒ Fee Transmittal Form	**ACCOMPANYING APPLICATION PARTS**
2. ☒ Specification and Drawings [Total Pages 28] - Specification - (20 pages) - Claims - (6 pages) - Abstract - (1 pages) - Sheets of Drawings - (1 sheet) ☐ Formal ☒ Informal	7. ☐ 37 CFR 3.73(b) Statement (when there is an assignee) ☐ Power of Attorney
	8. ☐ English Translation Document *(if applicable)*
	9. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
3. ☒ Oath or Declaration [Total Pages 2] a. ☐ Newly executed (original) b. ☒ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional with Box 17 completed)* ***[Note Box 4 below]***	10. ☒ Preliminary Amendment ☐ Drawings [Total Sheets] ☐ Letter to Official Draftsperson Including Drawings [Total Pages]
	11. ☒ Return Receipt Postcard
4. ☒ Incorporation by Reference (usable if Box 3b is checked) The entire Disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 3b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.	12. ☐ Small Entity Statement(s) ☐ Statements filed in prior application, (Status still proper and desired)
	13. ☐ Certified Copy of Priority Document(s)
5. ☐ Microfiche Computer Program *(Appendix)*	14. ☐ Deletion of Inventor(s) Signed statement attached deleting inventor(s) named in the prior application.
6. ☐ Nucleotide and/or Amino Acid Sequence Submission ☐ Computer Readable Copy ☐ Paper Copy (identical to computer copy) ☐ Statement verifying identify of above copies	15. ☐ Patent Application Data Entry Form 16. ☐ Other:

17. ☒ **If a CONTINUING APPLICATION,** check appropriate box and supply the requisite information:
☒ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application USSN 08/886,088, filed July 2, 1997, which is a continuation of USSN 07/995,331, filed December 23, 1992, now U.S. Patent No. 5,714,389, which is a continuation of USSN 07/702,450, filed May 16, 1991, which is a continuation of USSN 07/211,582, filed June 27, 1988.
Priority to the above application(s) is claimed under 35 U.S.C. 120.
Prior application information: Examiner: P. Do Group/Art Unit: 1641.

18. ☐ **Priority - 35 U.S.C. 119**
☐ Priority of application Serial No. _____ filed on _____ in _____ is claimed under 35 U.S.C. 119.
☐ The certified copy has been filed in prior U.S. application Serial No. _____/_____ on _____.
☐ The certified copy will follow.

CORRESPONDENCE ADDRESS	SIGNATURE BLOCK
Direct all correspondence to: Patent Administrator Testa, Hurwitz & Thibeault, LLP High Street Tower 125 High Street Boston, MA 02110 Tel. No.: (617) 248-7000 Fax No.: (617) 248-7100	Respectfully submitted, D. A. Greenhalgh Date: November 17, 1999 Reg. No. 38,678 Tel. No.: (617) 248-7317 Fax No.: (617) 248-7100 Duncan A. Greenhalgh Atty/Agent for Applicant(s) Testa, Hurwitz & Thibeault, LLP High Street Tower 125 High Street Boston, MA 02110

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PATENT
Atty. Docket No. CWP-012CN3
(1451/2)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

SERIAL NO.: _____ *(Continuation of U.S.S.N. 08/886,088)*

FILED: *Herewith*

TITLE: *TEST DEVICE AND METHOD FOR COLORED PARTICLE IMMUNOASSAY*

GROUP NO.: 1641
(Prior Application)

EXAMINER: P. Do
(Prior Application)

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Prior to substantive examination, please amend the application as follows:

In the Specification:

On page 1, immediately after the title "TEST DEVICE AND METHOD FOR COLORED PARTICLE IMMUNOASSAY" and before the title "BACKGROUND OF THE INVENTION" insert the following:

-- REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Serial No. 08/886,088, filed July 2, 1997, which is a continuation of U.S. Serial No. 07/995,331, filed December 23, 1992, now U.S. Patent No. 5,714,389, which is a continuation of U.S. Serial No. 07/702,450, filed May 16, 1991, now

abandoned, which is a continuation of U.S. Serial No. 07/211,582, filed June 27, 1988, now abandoned. --

Page 1, last line, delete "changes" and replace with -- change --.

Page 2, last line, delete "drawing" and replace with -- drawings --.

Page 12, line 23, delete "upstream" and insert -- downstream --.

Page 17, line 5, insert -- 10 -- after casing.

Page 17, line 7, delete "10" and insert -- 10' --.

Page 17, line 8, delete "10'" and insert -- 10" --.

Page 17, line 9, delete "10 and 10'" and insert -- 10' and 10" --.

Page 17, line 10, delete "10'" and insert -- 10" --.

Page 17, line 19, delete "10" and insert -- 10' --.

Page 17, line 21, delete 10 and insert -- 10' --.

Page 17, line 22, delete "10'" and insert -- 10" --.

In the Claims:

Cancel claims 1-13 without prejudice to their subsequent reintroduction into this application or their introduction into a subsequently filed continuation application.

REMARKS

This application is a continuation of U.S. Serial No. 08/886,088, filed July 2, 1997. Applicants have amended the specification to correct minor typographical errors and to update the specification to include a reference to related applications. In addition, Applicants have submitted a copy of the same Abstract, labeled page 27, that was submitted to the Patent Office during prosecution of the parent application, USSN 08/886,088.

Applicants believe that the aforementioned amendments introduce no new matter. In addition, Applicants believe that no addition fee is incurred by the submission of this paper.

Respectfully submitted,

Dated: November 17, 1999

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TEST DEVICE AND METHOD FOR COLORED PARTICLE IMMUNOASSAY

BACKGROUND OF THE INVENTION

This invention relates to assays for ligands, e.g., antigens, in a liquid sample such as a body fluid. More particularly, the invention relates to a method and apparatus for the detection of a ligand in a body fluid such as urine using a conjugate comprising colored particles and a novel flow-through test cell.

Many types of ligand-receptor assays have been used to detect the presence of various substances, often generally called ligands, in body fluids such as urine. These assays involve antigen antibody reactions, synthetic conjugates comprising radioactive, enzymatic, fluorescent, or visually observable metal sol tags, and specially designed reactor chambers. In all these assays, there is a receptor, e.g., an antibody, which is specific for the selected ligand or antigen, and a means for detecting the presence, and often the amount, of the ligand-receptor reaction product. Most current tests are designed to make a quantitative determination, but in many circumstances all that is required is a positive/negative indication. Examples of such qualitative assays include blood typing and most types of urinalysis. For these tests, visually observable indicia such as the presence of agglutination or a color changes are preferred.

Even the positive/negative assays must be very sensitive because of the often small concentration of the ligand of interest in the test fluid. False positives can also be troublesome, particularly with agglutination and other rapid detection methods such as dipstick and color change tests. Because of these problems, sandwich assays and other sensitive detection methods which use metal sols or other types of colored particles have been developed. These techniques have not solved all of the problems encountered in these rapid detection methods.

It is an object of this invention to provide a rapid, sensitive method for detecting ligands in body fluids. Another object is to provide an assay which has high sensitivity and fewer false positives than conventional assays. A further object is to provide a test cell for detection of low levels of ligands in body fluids. Another object is to provide an assay system which involves a minimal number of procedural steps, and yields reliable results even when used by untrained persons.

These and other objects and features of the invention will be apparent from the following description, drawing, and claims.

SUMMARY OF THE INVENTION

The invention features a method and test cell for the detection of a preselected ligand in a liquid sample such as a body fluid.

The test cell useful in the practice of the invention has an elongate outer casing which houses an interior permeable material, e.g., glass fiber, capable of transporting an aqueous solution by capillary action, wicking, or simple wetting. The casing defines a sample inlet, and interior regions which, for ease of description, can be designated as a test volume and a reservoir volume. The reservoir volume is disposed in a section of the test cell spaced apart from the inlet, and preferably is filled with sorbent material. The reservoir acts to receive liquid transported along a flow path defined by the permeable material and extending from the inlet and through the test volume. In the test volume is a test site comprising a first protein having a binding site specific to a first epitope of the ligand immobilized in fluid communication with the flow path, e.g., bound to the permeable material or to latex particles entrapped in or bonded to the permeable material. A window such as a hole or transparent section of the casing permits observations of the test site through the casing wall.

In a preferred embodiment, the flow path is restricted or narrowed in the test area, thereby channeling and concentrating fluid flow into contact with the test site. It is also preferred that the test cell include a solution filtering means disposed in the flow path between the sample inlet and the

test site. The filtration means can comprise a separate, conventional filter element disposed within the casing of the test cell in fluid communication with the permeable material defining the flow path, but preferably is defined simply by a portion of the permeable material itself. The provision of such a filtration means in the test cell has the effect of removing by entrapment from impure samples, such as urine samples, a portion of the particulates and nonspecific interfering factors which sometimes cause false positive readings.

The method of the invention requires the use of a conjugate comprising a second protein bound to colored particles such as a metal sol or colloid, preferably gold. The conjugate can take two distinct forms, depending on whether the assay is designed to exploit the "sandwich" or "competitive" technique.

In the case of the sandwich technique, the second protein comprises a site which binds to a second epitope on the ligand. This type of conjugate reacts with the ligand to form a complex in the liquid sample. The complex is detected by visual observation of color development at the test site in the test cell. At the test site, the ligand bound with the conjugate reacts with the immobilized first binding protein to form a "sandwich" of the first protein, ligand, second protein, and colored particles. This sandwich complex is progressively produced at the test site as sample continuously passes by, filling the reservoir. As more and more conjugate is immobilized, the colored particles

aggregate at the test site and become visible through the window, indicating the presence of ligand in the liquid sample.

In the case of the competitive technique, the second protein binds with the first protein in competition with the ligand. The second protein comprises, for example, an authentic sample of the ligand or a fraction thereof which has comparable affinity for the first protein. As the liquid sample is transported in contact with the test site, ligand, if any, and the conjugate compete for sites of attachment to the first protein. If no ligand is present, colored particles aggregate at the test site, and the presence of color indicates the absence of detectable levels of ligand in the sample. If ligand is present, the amount of conjugate which binds at the test site is reduced, and no color, or a paler color, develops.

In one embodiment of the invention, the test liquid is mixed with the conjugate outside the test cell. In another embodiment, the conjugate is disposed in freeze-dried or other preserved form on the permeable material between the inlet and the test site, and the sample liquid resolubilizes the conjugate as it passes along the flow path.

Color development at the test site may be compared with the color of one or more standards or internal controls to determine whether the development of color is a true indication of the presence or absence of the ligand, or an artifact caused by nonspecific sorption.

In one embodiment employing the sandwich technique, the standard consists of a negative control site, preferably disposed adjacent the test site, and visible through a second window proximate the first. The negative control site preferably is prepared identically to the test site, except immobilization of the first binding protein is omitted. Therefore, although the conjugate will reach the control site, it aggregates due only to non-specific binding. If the test site is not appreciably more intense in color than the control site, the assay is considered negative.

In another embodiment, the assay and test cell may include a positive control. Thus, when exploiting the sandwich technique, the cell may have an authentic sample of the ligand immobilized at a control site. If no color develops at this control site, the assay is considered inconclusive. When exploiting the competitive technique, the development of color at the positive control site means the assay results are inconclusive.

Broadly, the method of the invention involves the use of a test cell of the type described above to achieve an easily readable, sensitive, reproducible indication of the presence of a ligand, e.g., human chorionic gonadotropin (hCG), in a test sample such as a human urine sample. The method involves the step of transporting the sample and a conjugate comprising a protein bound to a metal sol or other colored particle along a flow path and in contact with a test site comprising immobilized binding protein specific to an epitope of the ligand,

and preferably also in contact with a control site. Preferably, the colored particle comprises a gold sol; the flow path in the region of the test site is reduced in cross-section relative to other parts of the flow path; the sample is passed through a filtration means after it enters the test cell but before it contacts the test site; and the test site comprises latex particles entrapped or otherwise fixed in the flow path having the immobilized protein on their surface. In the practice of the process, either the conjugate is premixed with the sample, or the conjugate is disposed in preserved form, e.g., lyophilized, in the flow path between the inlet and the test site. In either case, placement of the test cell in the sample, or application of the sample to the inlet, initiates flow, and the result is read by observing color development at the test site, or by comparing the color of the test site and control site.

The use of the colored particle detection system in combination with the filtration means, the concentrating effect of flow of the sample, and the ease of comparison between the colors of the test and control sites, together enable construction of a family of extremely sensitive assay systems which minimize false positives and can be used effectively by untrained persons.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 is a cut-away, schematic, top view of an embodiment of a test cell useful in explaining the test cell and process of the invention;

Figure 2 is a cross-sectional side view of the test cell of Figure 1;

Figure 3 is a perspective view of a currently preferred test cell constructed in accordance with the invention;

Figure 4A is a cross-sectional, top view of the test cell of Figure 3;

Figure 4B is a cross-sectional, side view of the test cell of Figure 3 taken at line 4B-4B of Figure 4A;

Figure 5 is a cross sectional view of the cell of Figure 3 taken at line 5-5 of Figure 4B; and

Figure 6 is a perspective view of another embodiment of a test cell constructed in accordance with the invention.

Like reference characters in the respective drawn figures indicate corresponding parts.

DESCRIPTION

The invention provides a test cell for conducting a sandwich or competitive immunoassay, and a process which utilizes the test cell and a conjugate comprising colored particles. As disclosed below, various features of the process and test cell of the invention cooperate to enable untrained personnel reliably to assay a liquid sample for the presence of extremely small quantities of a particular ligand while avoiding false positives and simplifying test procedures. The invention is ideal for use in over-the-counter assay test kits which will enable a consumer to self diagnose, for example, pregnancy, venereal disease, and other disease, infection, or clinical abnormality which results in the presence of an antigenic marker substance in a body fluid, including determination of the presence of metabolites of drugs or toxins. The assay process and the cell are engineered specifically to detect the presence of a preselected individual ligand present in a body or other fluids.

Broadly, the test cell and process of the invention can be used to detect any ligand which has heretofore been assayed using known immunoassay procedures, or known to be detectable by such procedures, using polyclonal or monoclonal antibodies or other proteins comprising binding sites for ligands. Various specific assay protocols, reagents, and analytes useful in the practice of the invention are known per se, see, e.g., U.S. 4,313,734, columns 4-18, and U.S. 4,366,241, columns 5-40.

The combination of features believed to be responsible for the excellent sensitivity and

reproducibility of assays constructed in accordance with the invention is the use of the novel test cell which serves to concentrate ligand from a test sample at a test site in the cell, and the use of a metal sol or other colored particle as a marker system which permits direct visual observation of color development. False positives are reduced while maintaining excellent sensitivity by including in the test cell a negative control or control site whose color is compared with the test site, and by including a filtration means which limits the introduction to the test site of contaminants from the sample.

The assay is conducted by simply placing the inlet of the test cell in contact with a liquid test sample. One then merely waits for the test sample to pass through the cell and into reactive contact with the test site (and optionally one or more control sites) visible through a window or windows in the cell's exterior casing. In one embodiment, the conjugate is mixed with the sample and incubated briefly before the test cell is inserted. In another embodiment, the conjugate is disposed in preserved form in the flow path within the cell. If the ligand is present in the sample, it passes through the inlet and the interior of the cell along the flow path past the test and control sites, where, in the sandwich embodiment, it reacts with immobilized binding protein, e.g., antibody, at the test site, and perhaps also non-specifically at the control site. A "sandwich" forms at the test site comprising immobilized binding protein-ligand binding protein-colored particle. The presence of the

sandwich complex and thus the ligand is indicated by the development of color caused by aggregation of the metal sol particles at the test site. A deeper color at the test site than at the negative control site is a positive indication of the presence of the ligand.

By providing a reservoir of sorbent material disposed beyond the test and control sites, a relatively large volume of the test liquid and any ligand it contains can be drawn through the test area to aid sensitivity. Optionally, the region of the flow path in the test cell defining the test and control sites is restricted in cross-sectional area relative to other regions of the flow path. This feature produces a "bottle-neck" effect wherein all ligand in the entire volume of sorbed sample must pass through the restricted flow area immediately about the test site where it will be immobilized by reaction with binding protein.

From the foregoing, it will be apparent that the success of the test procedure is dependent on ligand present in the sample reacting with the conjugate, or on reproducible competition between the ligand and the conjugate for sites of attachment at the test site. In accordance with the invention, as noted above, the assays can be conducted by premixing the conjugate with the liquid sample prior to introduction into the elongate test cell. Alternatively, the conjugate may be disposed in preserved form, e.g., freeze-dried, in the flow path within the test cell upstream of the test and control sites. In this case, the cell is placed directly in the liquid sample solution without premixing. Ligand, if any, passing up through the cell and entrained within the liquid moves into contact with

the conjugate forming an immune complex or initiating competition in situ as flow continues. This latter technique has the advantage that it eliminates a manipulative step in the assay procedure, and accordingly a possible source of error.

Referring to the drawing, figures 1 and 2 illustrate schematically an embodiment of a test cell 5 constructed in accordance with the invention useful in explaining its principles of construction. It comprises an outer, molded casing 10 which defines a hollow, elongate enclosure filled with a permeable, sorbent material 12. Casing 10 also defines a test liquid inlet 14 and a pair of circular openings 16, 18 comprising windows through which sorbent material 12 is visible.

Sorbent material 12 and the interior of casing 10 together define a flow path passing generally from left to right in figures 1 and 2. When the test cell is placed with inlet 14 disposed within or otherwise in contact with a liquid sample, the liquid is transported by capillary action, wicking, or simple wetting along the flow path through upstream flow section 20, test volume 22, and into reservoir volume 24, generally as depicted by the arrows. The flow section 20 of the flow path disposed inwardly of the inlet 14 serves as a filter which can remove from impure test samples particulate matter and interfering factors. The provisions of such a filtration means 20 downstream of the inlet 14 is believed to contribute to the success of the system and its ability to avoid false positives.

Disposed within sorbent material 12 is a band 26 of dehydrated conjugate, e.g., antibody-metal sol. As the liquid sample moves past band 26, the conjugate is entrained in the liquid, reconstituted, and reacts or competes with ligand, if present, dissolved in the liquid sample. Of course, conjugate band 26 may be eliminated, and the conjugate added to the test liquid prior to introduction of the cell 5 as previously noted.

Within the volume of sorbent material 12 disposed directly beneath circular openings 16 and 18 in casing 10 is disposed, respectively, control site 16' and test site 18'. In the drawing, the control and test site are illustrated as being disposed serially along the flow path. Alternatively, the control and test site or sites may be disposed side by side or in other spacial relationships.

Test site 18' comprises a preselected quantity of antibody against an epitope of the ligand to be detected immobilized in place within the flow path. Its detailed chemical structure can vary widely. Control site 16' is preferably identical in size and chemical makeup to test site 18', excepting that the immobilized antibody present at the test site 18' is omitted at the control site 16'. Thus, any nonspecific aggregation of, e.g., ligand-conjugate or free conjugate, which occurs at test site 18' also will occur at control site 16'. A deeper color at test site 18' as compared with control site 16' is a positive indication of ligand in the sample in the sandwich assay.

The invention is not limited by the precise nature of the test site 18' and corresponding control

site 16', and in fact, control site 16' may be entirely eliminated if a reduction in sensitivity can be tolerated. Generally, antibody or other binding protein may be immobilized at test site 18' using adsorption, absorption, or ionic or covalent coupling, in accordance with methods known per se. A currently preferred formulation for test site 18' is to immobilize monoclonal antibody against an epitope of the ligand on latex beads, and then to entrap or otherwise link the beads in sorbent material 12 at region 18'. Control site 16' is fabricated identically, except that the latex beads contain non specific immunoglobulin, e.g., immunoglobulin from bleedings from an animal that has not been immunized.

Disposed beyond test volume 22 is a reservoir volume 24 comprising a relatively large mass of sorbent or supersorbent material. The purpose of reservoir volume 24 is to assure that a reasonably large amount of test liquid is drawn through test volume 22. Increasing the volume of reservoir 24 can have the effect of increasing the sensitivity of the assay procedure, as it results in an increase in the amount of ligand passing through the test area 22. Suitable sorbents include commercial materials of the type available, for example, from The Dow Chemical Company of Midland, Michigan, and the Chemical division of American Colloid, Arlington Heights, Ill. These materials can absorb many times their weight in water and are commonly used in disposable diapers. They comprise lightly crosslinked polyacrylate salts, typically alkali metal salts.

Polyclonal antisera and indeed monoclonal antibodies or fractions thereof having specific binding properties and high affinity for virtually any antigenic substance are known and commercially available or can be produced from stable cell lines using well known cell fusion and screening techniques. The literature is replete with protein immobilization protocols. See, for example, Laboratory Techniques in Biochemistry and Molecular Biology, Tijssen, Vol. 15, Practice and Theory of Enzyme immunoassays, chapter 13, The Immobilization of Immunoreactants on Solid Phases, pp. 297-328, and the references cited therein.

Metal sols and other types of colored particles useful as marker substances in immunoassay procedures are also known per se. See, for example, U.S. 4,313,734, February 2, 1982, to Leuvering, the disclosure of which is incorporated herein by reference. For details and engineering principles involved in the synthesis of colored particle conjugates see Horisberger, Evaluation of Colloidal Gold as a Cytochromic Marker for Transmission and Scanning Electron Microscopy, Biol. Cellulaire, 36, 253-258 (1979); Leuvering et al, Sol Particle Immunoassay, J. Immunoassay 1 (1), 77-91 (1980), and Frens, Controlled Nucleation for the Regulation of the Particle Size in Monodisperse Gold Suspensions, Nature, Physical Science, 241, pp. 20-22 (1973).

The cell can take various forms. It will usually comprise an elongate casing comprising interfitting parts made of polyvinyl chloride, polypropylene, or other thermoplastic resin. Its interior flow path will contain a relatively inert

material or a combination of materials suitable for transporting the liquid. In some circumstances it may be preferable to use a material of higher sorptivity as the reservoir, promoting the flow of liquid, and a different material for remaining portions of the flow path.

From the foregoing it should be apparent that the advantages in reproducibility, sensitivity, and avoidance of false positives of assay systems constructed in accordance with the invention are traceable to a combination of features of the invention. In use, the test cell of the invention and the metal sol particles used as a marker together cooperate to result in an increase in color intensity progressively as ligand complexed with conjugate is trapped at the test site by the immobilized binding protein. This approach can be utilized to design assays and test cells for essentially any antigenic material.

The invention will be further understood from the following non-limiting examples.

Example 1

The currently preferred test device embodying the invention is shown in Figures 3, 4A, 4B, and 5. A modification of the device depicted in Figure 3 is shown in Figure 6, and includes a second control site 19 in addition to control site 16' and test site 18', as well as a stand 21 useful for maintaining the test cell in an incline position with the reservoir downhill. When a test sample is applied to inlet 14, gravity as well as sorption aids in transporting the sample along the flow path.

As shown in Figures 3, 4A, 4B, and 5, the preferred test cell of the invention differs from the exemplary device discussed above and shown in Figures 1 and 2 in certain of its more specific internal features. Specifically, the casing comprises a pair of interfitting polymeric parts including a U-shaped top part 10 which, when the device is assembled, interfits with lower part 10'. Top and bottom parts 10 and 10' may be connected through a hinge region 11. The bottom section 10' defines a pair of channels 28 above which is disposed a strip of glass fiber paper 13 (available commercially from Eaton Dikeman, Grade 111, or Whatman, Grade GFA). Test liquid applied through inlet 14 soaks along the paper strip 13 which defines the flow path and a filtering means region 20, as well as a positive control site 16' and test site 18' visible through windows 16 and 18 consisting of openings through upper mating member 10. The paper strip 13 overlaps into reservoir volume 24, which is defined by a cavity between the interfitting top and bottom mating members 10 and 10'. The cavity in this case is filled with sorbent blotting paper 12 comprising the sorbent reservoir. A suitable paper is sold as Grade 12A absorbent paper, a cellulose product available from Schleicher and Schuell. In one preferred embodiment, the dimensions of the glass fiber paper 13 were approximately one quarter inch by three inches, and those of the absorbent material 12 approximately two inches by five thirty seconds of an inch on each side. A number of these devices were produced and further treated to adapt them to detect pregnancy by assay of urine.

Test site 18' in each device was fabricated as a spot within fiber paper 13 using the following technique. Latex beads available commercially and comprising polystyrene particles 0.3 micron in diameter were passively coated with purified rabbit anti-human chorionic gonadotropin. The polyclonal antibody was purified using conventional techniques from bleedings of a rabbit previously immunized with human chorionic gonadotropin in a manner know per se. Equal parts of a latex (0.6% by weight) having a continuous phase of glycine buffer, pH = 8.3, and a 1 mg/ml antibody solution in the same buffer were mixed and incubated at 37°C. 15 microliters of this solution, comprising approximately 0.6% solids, were added, one drop at a time, to the glass fiber paper 13 to produce spot 18' after the devices had been assembled. The spots were then allowed to dry at 37°C. The control site 16' was produced identically to the test site disclosed immediately above, excepting that rabbit polyclonal non-immune gamma globulin was used in place of the anti-hCG gamma globulin.

Metal sol particles were prepared in accordance with the method of Frens, Controlled Nucleation for the Regulation of the Particle Size in Mono Dispersed Gold Solutions (1973), supra. Briefly, the gold sol was prepared by reducing a 4% solution of gold chloride with 1% sodium citrate to produce gold particles of approximately 18nm in diameter. The particles were made immunochemically reactive by admixture with a monoclonal antibody specific for human chorionic gonadotropin with no

detectable cross-reactivity with human leutinizing hormone. The antibody was purchased from Charles River Labs, and is produced using standard techniques including purification from ascites using HPLC ion exchange chromatography. It was added to the gold sol as a 10 ug/ml solution in borate buffer, pH-6. The bound antibody fraction is separated from the free fraction by either density centrifugation or gel filtration chromatography. Additional details of the currently preferred procedure for making the antibody sol conjugate are disclosed by Leuvering et al, J. Immunoassay (1980) supra. Individual batches of the latex and the conjugate are titrated to optimize activity so that a suitable amount of latex is applied to the test site and a suitable amount of conjugate is used in conducting the test.

Test Protocol

To a 10 X 50 mm test tube of lyophilized gold sol antibody conjugate is added 0.5 ml urine sample containing a known quantities of hCG. The samples comprised hCG standards purchased from Sigma Chemical Company diluted in processed, hCG negative urine. The contents of the tube are mixed by shaking in a horizontal motion until the lyophilized antibody is dissolved. The device depicted in Figures 3-5 is then inserted into the tube, and the results are read after the entire fluid volume has been absorbed.

The results of this qualitative procedure are as follows:

mIU hCG	Color of Control Spot	Color of Reagent Spot
0	grey	grey
25	grey	pink hue
50	grey	pink
100	grey	rose
150	grey	rose
>150	grey	dark rose

The pink color clearly visible at 50 mIU of human chorionic gonadotropin means that the test can detect pregnancy one day after a missed menstrual period. In initial stages of testing, approximately 50 negative samples from various sources have been run with no false positives or even border-line cases. It is anticipated that the commercial device will have less than 1% false positives.

Non-limiting examples of materials which may be assayed in accordance with the invention in addition to the human chorionic gonadotropin noted above include human leutinizing hormone, progesterone, estrogen, and streptococcus.

Other embodiments are within the following claims.

What is claimed is:

1. A method of detecting a ligand in a liquid sample, the method comprising the steps of:

A. transporting along a flow path in a test cell a solution, including a liquid sample suspected to contain a ligand and a conjugate, into contact with a test site visible through a window in a wall of said test cell,

said test site having immobilized thereon a first protein having a binding site specific to a first epitope on the ligand,

said conjugate comprising colored particles coupled to a second protein selected from the group consisting of proteins having a binding site specific to a second epitope on the ligand and proteins which bind with said first protein in competition with the ligand, and

B. continuing transport of said solution to progressively produce at said test site a complex comprising said ligand for a time sufficient to visually determine through said window whether a color is developed at said test site.

2. The method of claim 1 wherein said test cell comprises a filtration means for filtering said liquid sample, said method comprising the additional step of transporting the sample through said filtration means before said sample contacts said test site.

3. The method of claim 1 wherein the cross-sectional area of said flow path is restricted about said test site whereby ligand is localized at said test site during flow of solution thereby.

4. The method of claim 1 comprising the additional steps of transporting said solution into contact with a control site visible through a window in a wall of said test cell and comparing the color of said test site and control site.

5. The method of claim 4 wherein said control site comprises a negative control site free of said first protein.

6. The method of claim 4 wherein said control site comprises a positive control site having immobilized thereon an authentic sample of said ligand.

7. The method of claim 1 comprising the step of mixing said conjugate with said liquid sample prior to step A.

8. The method of claim 1 wherein said conjugate is disposed in said flow path, said method comprising the additional step of transporting said liquid into solubilizing contact with said conjugate prior to contact with said test site.

9. The method of claim 1 wherein said first and second proteins comprise antibodies and at least one of said proteins is a monoclonal antibody.

10. The method of claim 1 wherein said first protein has a binding site specific to an epitope of human chorionic gonadotropin.

11. The method of claim 1 wherein said first protein has a binding site specific to an epitope of human progesterone.

12. The method of claim 1 wherein said second protein has a binding site specific to a second epitope on the ligand, and when said sample contains said ligand, the complex produced in step B comprises said ligand bound to both said first and second proteins, and color is produced by aggregation of said colored particles at said test site.

13. The method of claim 1 wherein said second protein binds with said first protein in competition with the ligand, and

when said sample contains said ligand, the complex produced in Step B comprises said ligand bound to said first protein, and

when said sample is free of said ligand, the complex produced in step B comprises said conjugate bound to said first protein, and color is produced by aggregation of said colored particles at said test site.

14. A test cell for detecting a ligand in a liquid sample, the test cell comprising

an elongate casing for housing a permeable material and defining a liquid sample inlet, a reservoir volume, a test volume interposed between said inlet and reservoir volume, and a window through said casing at said test volume,

permeable material capable of transporting an aqueous solution disposed within said casing and defining a flow path extending from said sample inlet through said test volume and into communication with said reservoir volume,

a first protein having a binding site specific to a first epitope on said ligand, said first protein being immobilized at a test site, disposed within said test volume in fluid communication with said flow path and visible through said window, and

a sorbent material in said reservoir volume for drawing liquid sample along said flow path and into contact with said test site.

15. The cell of claim 14 further comprising a liquid sample filtering means disposed in said flow path between said inlet and said test site.

16. The cell of claim 15 wherein said filtering means is defined by a portion of said permeable material.

17. The cell of claim 14 wherein the cross sectional area of said flow path is restricted about said test site so that ligand in liquid passing therealong is localized at said test site.

18. The cell of claim 14 wherein said casing defines a second window through said casing and said cell further comprises a control site in fluid communication with said flow path visible through said second window.

19. The cell of claim 18 wherein said control site comprises a negative control site free of said first protein.

20. The cell of claim 19 wherein said control site comprises latex particles disposed in contact with said permeable material.

21. The cell of claim 18 wherein said control site comprises a positive control site having immobilized thereon an authentic sample of said ligand.

22. The cell of claim 14 further comprising a conjugate disposed in said flow path between said test site and said inlet; said conjugate comprising colored particles coupled to a second protein selected from the group consisting of proteins having a binding site specific to a second epitope on the ligand, and proteins which bind with said first protein in competition with the ligand.

23. The cell of claim 14 wherein said test site comprises an antibody fixed to latex particles disposed in contact with said permeable material.

24. The cell of claim 14 wherein said first protein binds with an epitope of human chorionic gonadotropin.

25. The cell of claim 14 wherein said first protein binds with an epitope of human progesterone.

26. The cell of claim 22 wherein at least one of said first and second proteins is a monoclonal antibody.

Abstract of the Disclosure

Disclosed is a test cell and a method for detection of a preselected ligand in a liquid sample such as a body fluid. The test cell includes an elongate outer casing which houses an interior permeable material capable of transporting an aqueous solution and defining a sample inlet, a test volume, and a reservoir volume. The reservoir volume is disposed in a section of the test cell spaced apart from the inlet and is filled with sorbent material. The reservoir acts to receive liquid transported along a flow path defined by the permeable material and extending from the inlet and through the test volume. In the test volume is a test site which includes a first protein having a binding site specific to a first epitope of the ligand immobilized in fluid communication with the flow path. The test site can be observed through a window of the casing.

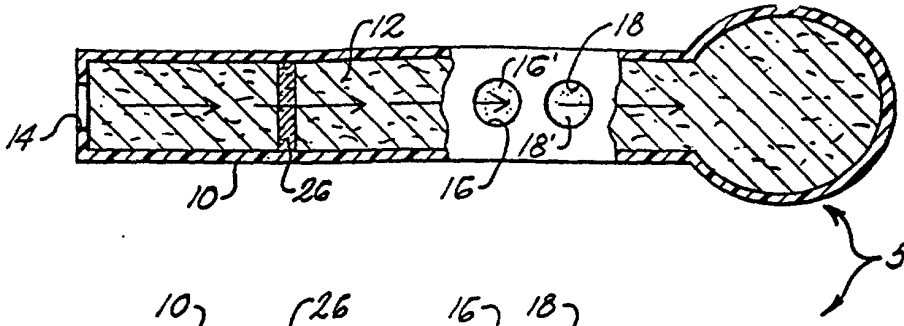

FIG. 1

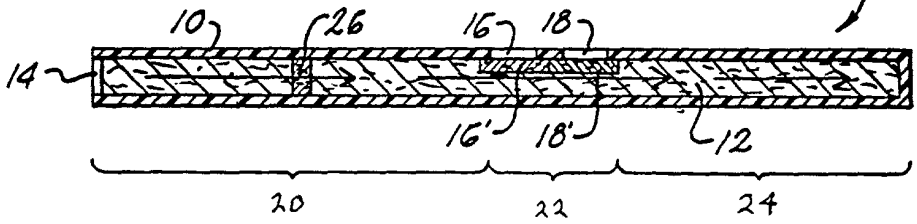

FIG. 2

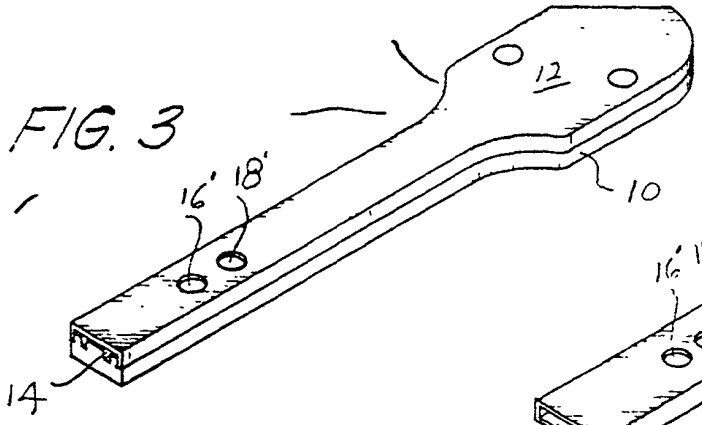

FIG. 3

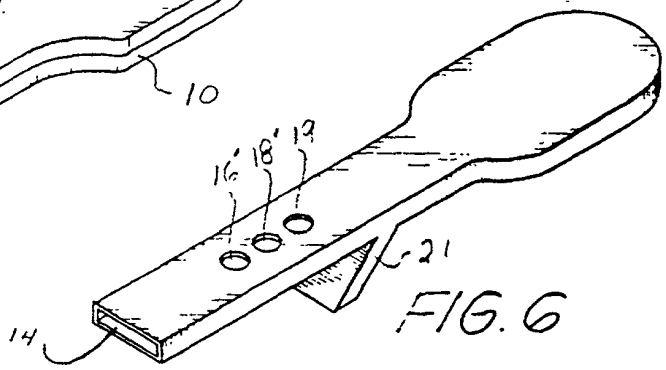

FIG. 6

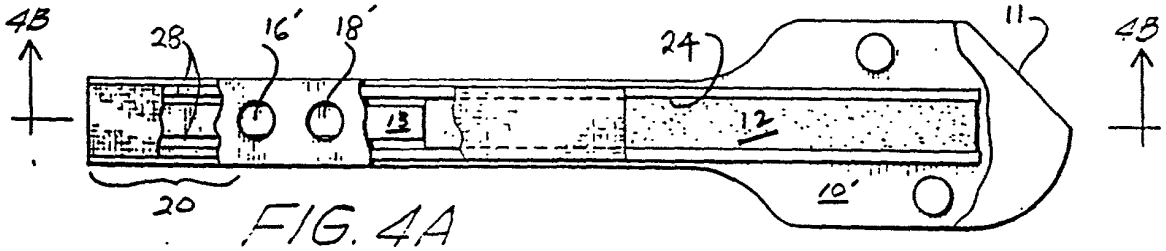

FIG. 4A

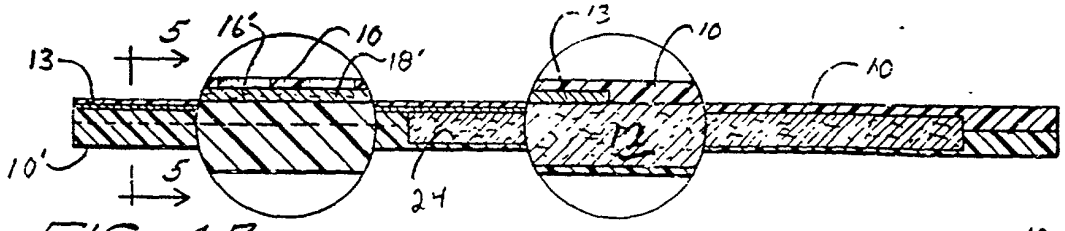

FIG. 4B

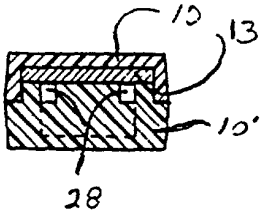

FIG. 5

EXPRESS MAIL MAILING LABEL

No. EL280659625US

COPY

Attorney's Docket Number CWP-012

Declaration, Petition and Power of Attorney For Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Test Device and Method for Colored Particle Immunoassay

the specification of which

(check one)

☒ is attached hereto.

☐ was filed on _____ as Application Serial No. _____ and was amended on _____ (if applicable).

I do not know and do not believe that the subject matter of this application was ever known or used in the United States before my invention thereof or patented or described in any printed publication in any country before my invention thereof or more than one year prior to the date of this application, and that said subject matter has not been patented or made the subject of an issued inventor's certificate in any country foreign to the United States on an application filed by me or my legal representatives or assigns more than twelve months prior to the date of this application; that I acknowledge my duty to disclose information of which I am aware which is material to the examination of this application, that no application for patent or inventor's certificate on the subject matter of this application has been filed by me or my representatives or assigns in any country foreign to the United States, except those identified below, and that I have reviewed and understand the contents of the specification, including the claims as amended by any amendment referred to herein.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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PATENT
Attorney Docket No. CWP-012CN3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S): Charlton et al.

SERIAL NO.: Continuation of USSN 08/886,088 GROUP NO.: 1641 (prior application)

FILED: November 17, 1999 EXAMINER: P. Do (prior application)

TITLE: TEST DEVICE AND METHOD FOR COLORED PARTICLE IMMUNOASSAY

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Box Patent Application
Assistant Commissioner for Patents
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Date: November 17, 1999
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